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# Subchronic Toxicity of Chlorine Dioxide and Related Compounds in Drinking Water in the Nonhuman Primate

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16. ABSTRACT

Subchronic toxicities of $ClO_2$, $NaClO_3$, and $NH_2Cl$ were studied in the African Green monkeys (Cercopithecus aethiops). The chemicals were administered in drinking water during 30-60 days subchronic rising dose protocols. The only unexpected and significant toxic effects was elicited by $ClO_2$; this chemical inhibited thyroid metabolism in the animals at a dose of ca.9.0 mg/kg/day. A statistically significant decrease of serum thyroxine occured after the fourth week of exposure to 100 mg/l.concentration. The extent of thyroid suppression was dose dependent in each individual monkey, and was reversible after cessation of exposure. $NaClO_2$ and $NaClO_3$ failed to elicit similar effects in doses up to ca. 60 mg/kg/day. Also, $NaClO_4$ or $NH_2Cl$ did not cause T-4 suppression in doses of 10 mg/kg/day. The selective thyroid effect of $ClO_2$ was unexplained and it appeared to be paradoxical since $ClO_2$ was rapidly reduced by the oral and gastric secretions to nonxidizing species (presumably $Cl^-$). No evidence of thyroid effects were detected in the serum of human volunteers who ingested~ 1 mg/l. of $ClO_2$ in drinking water as a result of routine use in the community water treatment process. Sodium Chlorite induced dose-dependent oxidative stress on hematopoesis, causing decreased hemoglobin and red cell count and increased methemoglobin content. At the same time, serum transaminase (SGPT) levels showed significant subclinical elevation. The hematologic effects of $NaClO_2$ rebounded during exposure indicating compensatory hemopoietic activity taking effect furing oxidative stress. Sodium and chlorate and chloramine did not detactable hematologic changes in the animals.

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# Subchronic Toxicity of Chlorine Dioxide and Related Compounds in Drinking Water in the Nonhuman Primate

by J. P. Bercz,* L. Jones,* L. Garner,* D. Murray,* D. A. Ludwig* and J. Boston*

**Subchronic toxicities of $ClO_2$, $NaClO_2$, $NaClO_3$ and $NH_2Cl$ were studied in the African Green monkeys (*Cercopithecus aethiops*). The chemicals were administered in drinking water during 30–60 days subchronic rising dose protocols. The only unexpected and significant toxic effect was elicited by $ClO_2$; this chemical inhibited thyroid metabolism in the animals at a dose of ca. 9.0 mg/kg/day. A statistically significant decrease of serum thyroxine occurred after the fourth week of exposure to 100 mg/l.concentration. The extent of thyroid suppression was dose dependent in each individual monkey, and was reversible after cessation of exposure. $NaClO_2$ and $NaClO_3$ failed to elicit similar effects in doses up to ca. 60 mg/kg/day. Also, $NaClO_4$ or $NH_2Cl$ did not cause T-4 suppression in doses of 10 mg/kg/day. The selective thyroid effect of $ClO_2$ was unexplained and it appeared to be paradoxical since $ClO_2$ was rapidly reduced by the oral and gastric secretions to nonoxidizing species (presumably $Cl^-$). No evidence of thyroid effects were detected in the serum of human volunteers who ingested ~ 1 mg/l. of $ClO_2$ in drinking water as a result of routine use in the community water treatment process.**

**Sodium chlorite induced dose-dependent oxidative stress on hematopoesis, causing decreased hemoglobin and red cell count and increased methemoglobin content. At the same time, serum transaminase (SGPT) levels showed significant subclinical elevation. The hematologic effects of $NaClO_2$ rebounded during exposure indicating compensatory hemopoietic activity taking effect during oxidative stress. Sodium chlorate and chloramine did not induce detectable hematologic changes in the animals.**

## Introduction

Owing to its excellent microbicidal properties, chlorine dioxide ($ClO_2$), a water-soluble yellow oxidant gas, has been used in the past for drinking water disinfection. The apparent relative absence of the carcinogenic trihalomethanes (THM) in $ClO_2$ treated water triggered renewed interest in this compound as a possible alternative to chlorine (*1*), since the latter was shown to generate THMs (reacting) with humic substances (*2*, *3*).

Concomitantly, the toxicology of $ClO_2$ and its metabolites ($ClO_2$ and $ClO_3$) have received wide attention in the recent literature. By using orally administered $ClO_2$, hematologic changes and inhibition in testicular uptake of $^3H$-thymidine was demonstrated in rats by Abdel-Rahman (*4*). Effects of these chlorine oxides on the glucose-6-phosphate dehydrogenase (G6PD)-deficient mouse were reported by Moore et al. (*5*). $ClO_2$ associated kinetics of red cell GSH depletion and intravascular hemolysis in rats and chickens was reported by Abdel-Rahman et al. (*6*). These workers also described the metabolism of $^{36}ClO_2$ (*7*) in rodents. In addition, the effects of $ClO_2$ and metabolites as they effect the cellular GSH system in the rat, mouse and chicken blood, were investigated by Couri et al. (*8*) Oxidative *in vitro* damage to erythrocytes by $NaClO_2$ was reported

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**Table 1. Clinical test protocol.**

| Test performed | Time of exposure, weeks | | | | | | Test substance[a] |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 8 | 16 (Rest) | |
| Red cell GGPD | X | | | | | | 1 |
| Red cell count and indices | X | X | X | X | X | | 5 |
| Reticulocytes | X | X | X | X | X | X | 5 |
| Osmotic fragility | X | | | | X | | 5 |
| Methemoglobin content | X | X | X | X | X | X | 5 |
| Hemoglobin content | X | X | X | X | X | X | 5 |
| White cell count and differential | X | X | X | X | X | X | 5 |
| Red cell GHS content | X | | X | | X | | 2, 3, 4 |
| Creatinine and BUN | X | X | X | X | X | X | 5 |
| Total bilirubin | X | X | X | X | X | X | 5 |
| Total protein, albumin | X | X | X | X | X | X | 5 |
| Alkaline phosphatase, LDH, SGOT, SGPT | X | X | X | X | X | X | 5 |
| Total T-4 | X | X | | X | | X | 5 |
| Body weight measurement | X | X | X | X | X | X | 5 |

[a]Test substances: 1 = $ClO_2$; 2 = $ClO_2^-$; 3 = $ClO_3^-$; 4 = $NH_2Cl$; 5 = all.

**Table 2. Hematological normal values for African Green monkeys.**

| Test | Sex | Normal range |
|---|---|---|
| Hemoglobin, g/dl | M | 16.2–19.03 |
| | F | 11.85–14.72 |
| Red cells × $10^6$/mm$^3$ | M | 6.15–7.1 |
| | F | 4.57–5.85 |
| White cells × $10^3$/mm$^3$ | M | 2.72–5.52 |
| | F | 3.78–8.65 |
| Hematocrit, % | M | 49.27–59.65 |
| | F | 36.26–44.26 |
| Reticulocytes, % | M | 0.34–2.35 |
| | F | 0.14–2.06 |
| Methemoglobin, % | M | 0.0–0.75 |
| | F | 0.0–0.75 |

by Heffernan et al. (*9*). The same authors also examined the *in vivo* effects of $NaClO_2$, demonstrating dose dependent oxidative stress in rats (*10*).

More recently, Michael et al. (*11*) of this laboratory published results of a prospective epidemiology study, showing the absence of clinicopathological effects in human volunteers to $ClO_2$ treatment of their community water supply. As an adjunct to the human study, we examined the subchronic toxicity of these chlorine oxides in nonhuman primates. Additionally we incorporated monochloramine ($NH_2Cl$) since this chemical is an ubiquitous component of chlorinated drinking water, often purposefully generated in the water treatment process.

## Materials and Methods

### The Experimental Design

Each of the chemicals examined were administered to the animal colony in exponentially rising step doses, each period lasting 30–60 days depending on the availability of scheduled resources for sampling and testing the blood samples. At each dose changeover the animals were bled, followed by bi-weekly repeated bleeding. (See Table 1 for testing schedule). Between chemicals the animals were rested for 6–9 weeks, at the end of which time base lines in clinical parameters were re-established. For purposes of statistics and evaluation, each animal served as its own control.

### The Animal Model

A small stable colony of African Green Monkeys (*Cercopithecus aethiops*) consisting of five adult males and seven adult females were used in the study. The body weights ranged from 3.0 to 5.7 kg, and their red cell G6PD activities ranged from 9.0–11.4 IU/g Hb with a mean of 8.8 IU/g Hb. All of the animals were in our possession for the past 8 years and repeated medical and laboratory examinations ascertained that the animals were in excellent health. Their hematological normal values are listed in Table 2. Repeated tuberculin tests and chest x-rays were performed throughout the study to assure the absence of mycobacterial infections. The animals were housed individually. They were fed a diet of Purina Monkey Chow supplemented daily with fresh fruit. During periods of exposure distilled water was made available *ad libitum*. For purposes of restraint light anaesthesia was induced by IM injection of ketamine HCl in uniform doses of 10.0 mg/kg.

### Test Solutions

A stock $ClO_2$ solution of 400–500 mg/l was prepared by purging $ClO_2$ from an acidified-$NaClO_2$

generator through an absorbent $NaClO_2$ solid column into 1 gallon quantities of distilled deionized water. The stock solution was then diluted to the appropriate concentrations (30, 100 and 200 mg/l) and was dispensed to the animals in dark bottles (2–3 liters/animal) equipped with ball-valve sipping tubes to minimize drippage. The solutions were changed, and consumption was measured three times weekly. Concentration and purity of the solutions were determined before administration and at the time of refilling to determine the extent of hydrolytic and photolytic degradation during residence in the bottle. Ultraviolet spectroscopy $E_{360_{nm}} = 1.1 \times 10^5$ mole$^{-1}$cm$^{-1}$) and titrimetry according to Palin (*12*) was employed for the assays. Absence of $Cl_2$ and of $OCl^-$ was verified by the $AgNO_3$ test for $Cl^-$. All consumption measurements were made by weight differential.

Chlorite and chlorate solutions (25, 50, 100, 200 and 400 mg/l. as ionic equivalents to $ClO_2$) were prepared from the corresponding analytical grade sodium salts. Monochloramine solutions were prepared according to Guion (personal communication). Dispensing and dosage of these solutions were as described above.

## Clinical Pathology Procedures

According to the experimental schedule shown in Table 1 blood was collected from the saphenous vein under light anaesthesia. Care was exercised not to draw more than 8 ml per bleeding period to avoid excessive blood loss and anemia.

Hematology tests (Table 1) were peformed according to standard procedures described by Wintrobe (*13*). For the determination of cell counts, cell indices and hemoglobin content a Coulter ZBI-6 cell counter and hemoglobinometer was used. Serum chemistries and enzyme activities were determined using a Union Carbide Centrifichem-400 kinetic analyzer. Red cell glucose-6-phosphate dehydrogenase (G6PD) was determined with the Calbiochem Reagent set using the Centrifichem procedure according to Favara (personal communication). Red cell glutathione (GSH) levels were determined manually by using the Biomedix DTNB reagent kit. Serum thyroxine (T-4) was determined with the SYVA enzyme amplified immunoassay (EMIT) reagent kit using the Centrifichem procedure as per SYVA's modifications. All chemistries, enzymes and T-4 determinations were made in duplicate. Quality assurance in the laboratory procedures was according to CAP standards, and quality control was achieved by using assayed and unassayed control specimen (normal, abnormal Coulter 4C, Dade Monitrol I and II).

**Table 3. *In vitro* inactivation of $ClO_2$ in saliva.[a]**

| Saliva: $ClO_2$ (mg/l.) | Recovery, % | |
|---|---|---|
| | Spectral | Titration |
| 1:9 (300) | 39 | 46 |
| 1:1 (300) | 26 | 28 |
| 1:9 (30) | 12 | 11 |
| 1:1 (30) | 5 | 4 |

[a]Reaction time = 1 min.

## *In Vitro* Deactivation of $ClO_2$ by Saliva

Pooled saliva was collected by buccal scraping from anaesthetized animals. The specimen was diluted 1:5 with distilled $H_2O$, and the resultant solution was used for the subsequent experiment. Various ratios of $ClO_2$ solutions were mixed with the dilute saliva in a quartz cuvet. The absorbance of the cuvet was read in a Perkin-Elmer ultraviolet spectrophotometer at 360 nm against a distilled $H_2O$ blank. At the same time another aliquot of the mixture was prepared and titrated according to Palin (*12*). Recovered concentrations were calculated using the molar extinction coefficient of $ClO_2$ of $1.1 \times 10^5$mole$^{-1}$cm$^{-1}$, and by titer equivalent. Reactant ratios and percent recoveries are summarized in Table 3.

## *In Vivo* Deactivation of $ClO_2$ in the Gastric Space

A 5.7 kg male animal was lightly sedated after an overnight fast and immobilized in a monkey-chair. The animal's stomach was intubated via the oropharyngeal route, and an aliquot of 30 ml $ClO_2$ (60 mg/l.) solution was instilled into the gastric space with a glass syringe. Immediately after discharging, a 15 ml aliquot of the solution was withdrawn, and its total oxidizing capacity (sum of $ClO_2$, $ClO_2^-$ and part of $ClO_3$) was determined (within 5 min) by iodometry at pH 1.0. Of the original $ClO_2$ equivalent oxidizing titer, 8% was recovered after 5 min of total contact time. Because of turbidity, spectrophotometry could not be used to quantitate unchanged $ClO_2$.

## Thyroid Function Test on Human Sera

Frozen serum samples from 350 human volunteers of the prospective epidemiology study previously reported by Michael et al. (*11*) were in our possession. Samples were selected on the basis of medical history of the volunteers. Only euthyroid

**Table 4. Response of clinical tests in nonhuman primates exposure to $ClO_2$, $NaClO_2$, $NaClO_3$ and $NH_2Cl$.**

| Test | Response | | | |
|---|---|---|---|---|
| | $ClO_2$ | $NaClO_2$ | $NaClO_3$ | $NH_2Cl$ |
| Red cell G6PD | NR | NR | NR | — |
| Red cell count | NR | D | SlD | NR |
| Cell indices | NR | D | SlD | NR |
| Reticulocytes | NR | SlI | NR | NR |
| Osmotic fragility | NR | NR | NR | — |
| Methemoglobin | NR | SlI | NR | NR |
| Hemoglobin | NR | SlD | SlD | NR |
| Leukocyte count | NR | NR | NR | NR |
| Differential count | NR | NR | NR | NR |
| GSH red cell | — | — | NR | NR |
| Creatinne and BUN | NR | NR | NR | NR |
| Total bilirubin | NR | NR | NR | NR |
| Total protein, albumin | NR | NR | NR | NR |
| Alkaline phosphatase, LDH | NR | NR | NR | NR |
| SGOT | NR | NR | NR | NR |
| SGPT | NR | I | NR | NR |
| Serum T-4 | D | SlD | NR | NR |
| Body weight | NR | NR | NR | NR |

[a]Response legend: NR = no response ($p < 0.01$); D = statistically significant decrease with dose dependence; I = statistically significant increase with dose dependence; SlI = slight increase with dose dependence; SlD = slight decrease with dose dependence; — = not done.

**Table 5. Effects of $ClO_2$, $NaClO_2$, $NaClO_3$ and $NH_2Cl$ on circulating total thyroxine in primates.**

| Test chemical | Serum T-4, μg/dl | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 mg/l. (4 wk) | 100 mg/l. (1 wk) | 100 mg/l. (6 wk) | 400 mg/l. (8 wk) | Rest |
| $ClO_2$ | 4.7 | 4.90[a] | 4.4 | 3.5[b] | — | 5.3 |
| $NaClO_2$ | 4.99 | | | | 4.43[c] | 5.3 |
| $NaClO_3$ | 5.3 | | | | 5.7[d] | 5.4 |
| NHCl | 5.28 | | | 5.8[e] | | |

Range of CV-s (Between Animals): 17 – 27%
Range of Precision (Between runs, Date Monitrol I Assayed Control Serum) 13.6% $\bar{X} = 5.66 \pm 0.77$
Quoted Range: 4.2 – 6.4

[a]Total body dose = 3.5 ± 0.9 mg/kg/day.
[b]Total body dose = 9.5 ± 5.8 mg/kg/day.
[c]Total body dose = 58.4 ± 27.6 mg/kg/day.
[d]Total body dose = 54.2 ± 38 mg/kg/day.
[e]Total body dose = 10 mg/kg/day.

**Table 6. Thyroid function in human population exposed to $ClO_2$ in drinking water.[a]**

| | $N$ | Serum T-4, meq/dl[b] |
|---|---|---|
| Exposed group, March | 115 | 7.59 ± 2.2 |
| Exposed group, June | 111 | 7.8 ± 2.4 |
| Control group, March | 79 | 7.67 ± 2.03 |
| Control group, June | 72 | 7.7 ± 2.2 |

[a]Only euthyroid volunteers included.
[b]As $\bar{X} \pm SD$.

individuals were included in the study. Thyroid status of individuals with known thyroidopathies were also confirmed. The method for thyroxine determination was identical to that used for the nonhuman primate samples.

## Results

Table 4 lists all clinical tests with annotations of dose responses to each chemical tested.

The thyroid hormone measurement results obtained with the monkey specimen are listed in Table 5, and the human T-4 values are summarized in Table 6. The oral doses of the four chemicals tested are also summarized in Table 6.

Figure 1 is a graphic representation of the thyroid inhibition regression in twelve animals versus mean doses of $ClO_2$ ingested. Each point signified by an X on the graph represents the $X/Y$ coordinate of the thyroxine shift versus the mean dose ingested by a single animal during the 4-week exposure to 100 mg/l. of $ClO_2$.

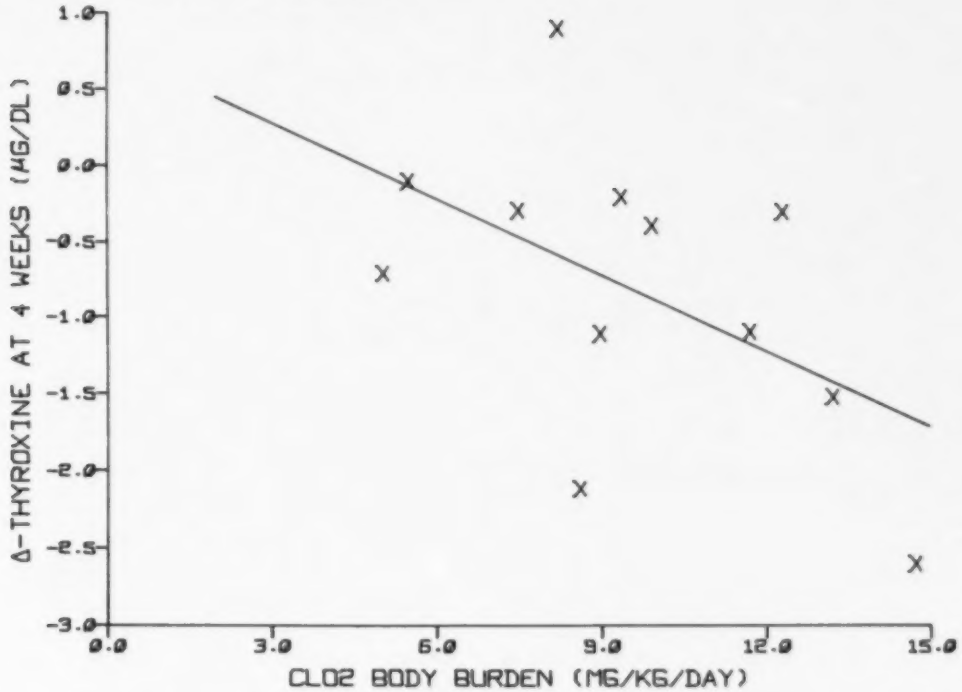


FIGURE 1. Correlation between thyroid depression and $ClO_2$ consumption at 4th week exposure to 100 ppm. Slope = 1.47; $y$ intercept = 0.86; $r = 0.53$; $t = 1.98$; $t(0.05) = 1.79$.

Figures 2 and 3 depict the chronological changes in hematologic parameters in male and female monkeys during $NaClO_2$ exposure. Figures 4 and 5 represent the temporal behavior of the hematologic values during $NaClO_3$ exposure.

Table 7 lists the mean SGPT and SGOT enzyme activities in 12 monkeys during the step dose $NaClO_2$ administration.

## Discussion

In view of the extent of hematologic oxidative effects and causative concentrations ranging up to 1000 mg/l. in rats and in other animals reported by Abdel-Rahman (*4*) and Moore (*5*), the apparent absence of hematologic and clinicochemical effects in the monkeys is not surprising. At the higher concentration stages of this study (100 and 200 mg/l.) the mean daily dose remained nearly constant at ca. 9 mg/kg/day. This observation reflects on the strong irritating nature of $ClO_2$ solutions. During the 200 mg/l. exposure, erythema and ulceration of the oral mucosa, mucous nasal discharge and avoidance of drinking water by the animals was observed. Throughout the $ClO_2$ study liquid consumption decreased with the strength of the solution. For example, at 0 mg/l. the mean water consumption was ca. 125 ml/kg/day with some seasonal fluctuations; at 100 mg/l. the consumption decreased to ca. 95 ml/kg/day, which further decreased to ca. 55 ml during the 200 mg/l. concentration. In fact, the high dose study was terminated after one week because some of the animals showed signs of dehydration and azotemia.

The most striking effect of $ClO_2$ was on the thyroid gland. At the ca. 9 mg/kg/day dose this chemical appeared to be a potent inhibitor of thyroid synthesis (Table 5). Analysis of the water consumption data at the 100 mg/l. exposure disclosed that the T-4 depression in each animal was statistically related to water consumption and $ClO_2$ dose (Fig. 1). Since the animals' fluid intake was near normal, the effect cannot be explained by dehydration. Adverse influence of ketamine-nitrous oxide anaesthesia on thyroid metabolism in human patients was published by Matsuki et al. (*14*). The possibility of ketamine-induced thyroid inhibition, however, must be discounted, since we followed identical ketamine anaesthesia protocols during each study of the four chemicals. One may also propose that $ClO_2^-$ and/or $ClO_3^-$, which were shown to be metabolites of $ClO_2$ (*6*), could inhibit iodine metabo-

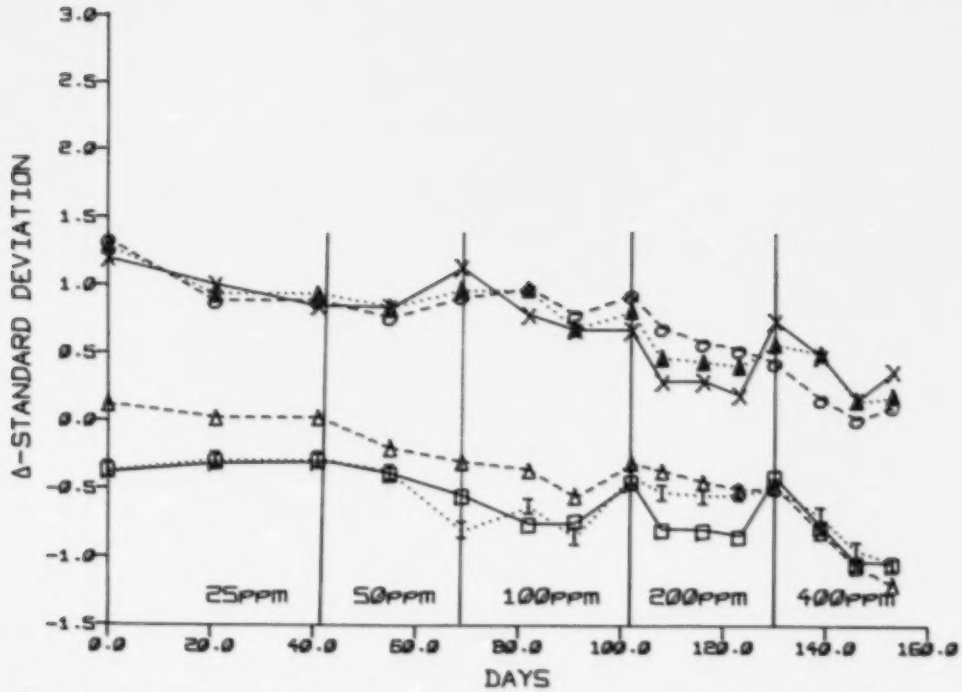


FIGURE 2. Effect of $NaClO_2$ on erythropoiesis of monkeys: (X) Hb males; (□) Hb females; (○) RBC males; (Δ) RBC females; (▲) Hct males; (I) Hct females.

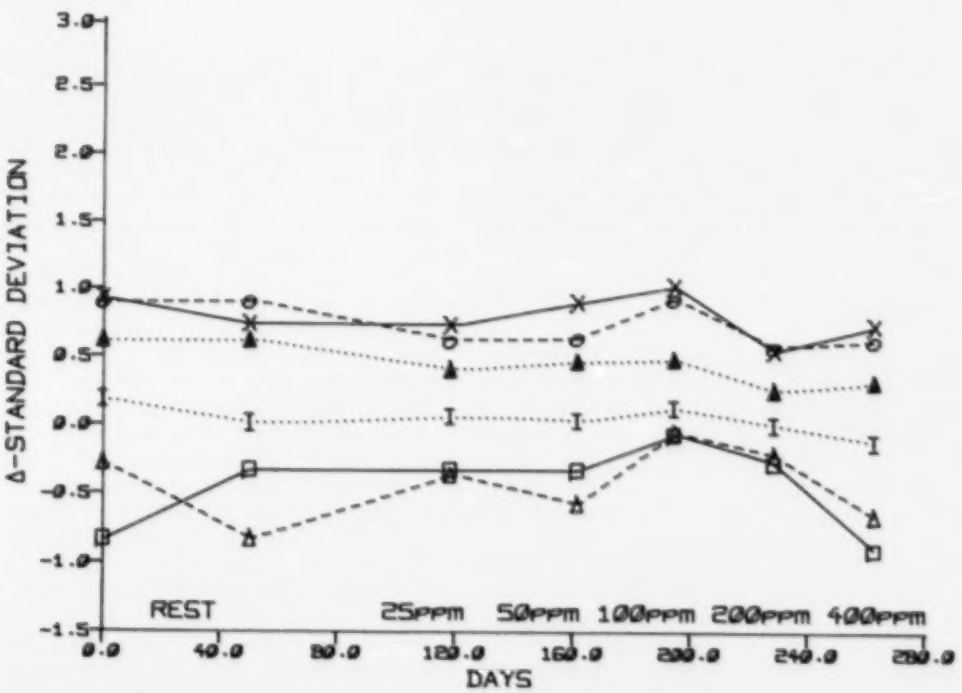


FIGURE 3. Effect of $NaClO_2$ on methemoglobinemia in monkeys: (X) reticulocytes males; (□) reticulocytes females; (○) methemoglobin males; (Δ) methemoglobin females.

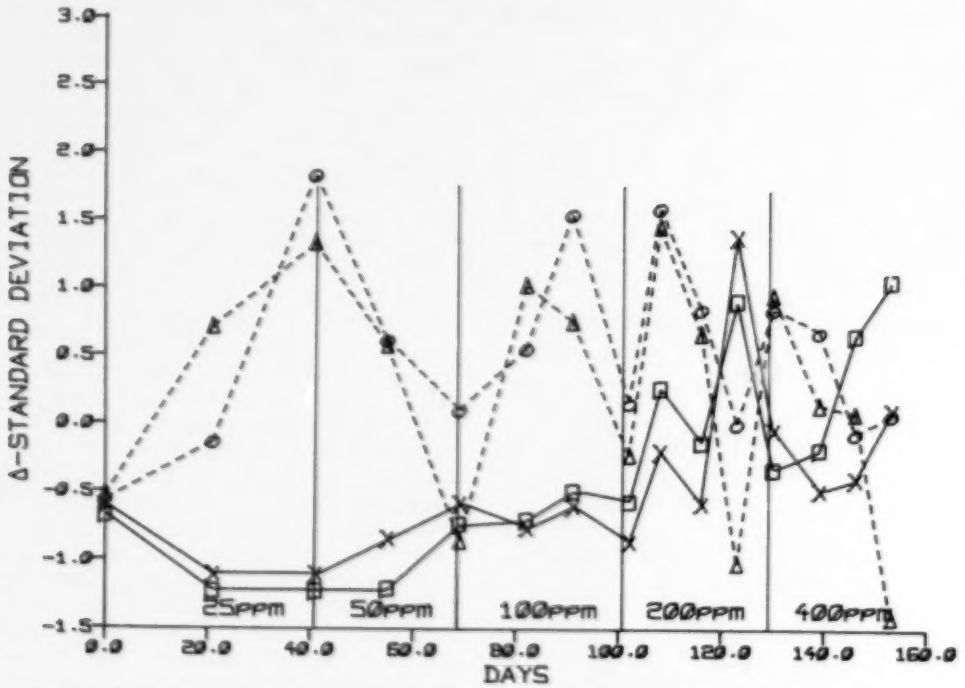


FIGURE 4. Effect of $NaClO_3$ on erythropoiesis in monkeys: (X) Hb males; (□) Hb females; (○) RBC males; (Δ) RBC females (▲) Hct males; (I) Hct females.

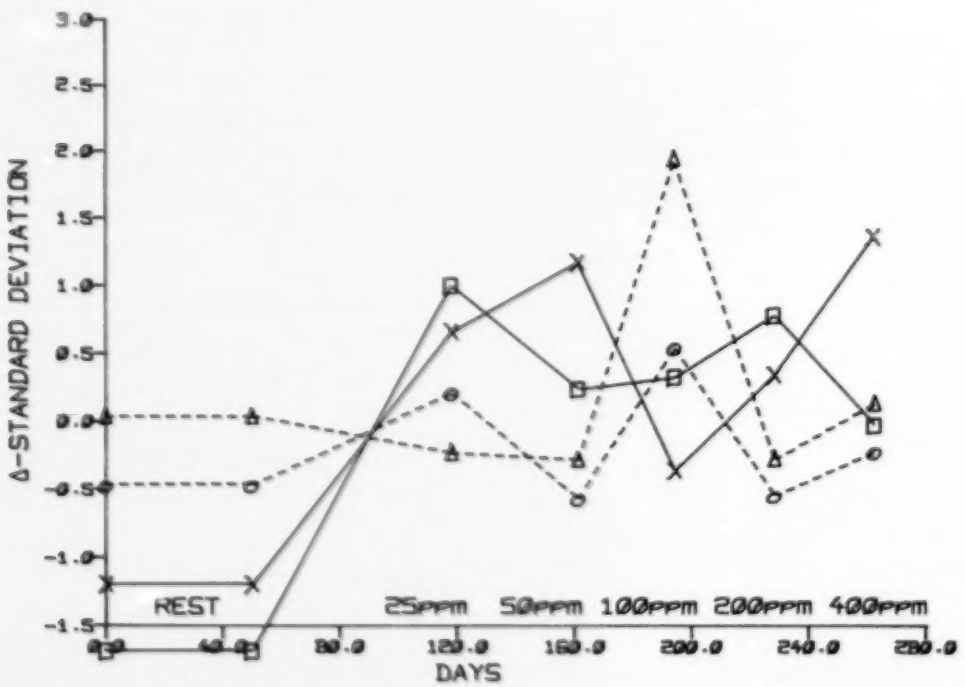


FIGURE 5. Effect of $NaClO_3$ on methemogloginemia in monkeys: (X) reticulocytes males; (□) reticulocytes females; (○) methemoglobin males; (Δ) methemoglobin females.

**Table 7. Mean AST and ALT activities in monkey serum during $NaClO_2$ exposure.**

| Dose, ppm | N | AST, IU/l. | ALT, IU/l. |
|---|---|---|---|
| 25 | 12 | 22.3 | 4.1 |
| 50 | 12 | 24.46 | 5.7 |
| 100 | 12 | 24.75 | 6.4 |
| 200 | 12 | 25.35 | 19.8 |
| 400 | 12 | 23.00 | 20.0 |

[a] $r^2 = 0.78$; $t = 3.28$; $t\ (0.05) = 2.35$.

lism in analogous fashion to the known goitrogenic perchlorate ion, (*15*, *16*) as shown in Eqs. (1)-(3).

$$ClO_2 + e^- \xrightarrow[\text{reduction}]{\text{Metabolism}} ClO_2^- \quad (1)$$

$$2\,ClO_2 + H_2O \xrightarrow[\text{hydrolysis}]{\text{Metabolism}} ClO_2^- + ClO_3^- \quad (2)$$

$$ClO_2^-, ClO_3^- \xrightarrow{\text{Absorption}} \text{Inhibition of } I^- \text{ metabolism within the thyroglobulin, analogous to } ClO_4^-. \quad (3)$$

This hypothesis is invalid because $NaClO_2$ and $NaClO_3$ failed to elicit thyroid inhibition in doses up to 60 mg/kg/day. Moreover, $NaClO_4$ administered in drinking water did not show antithyroid effects in 10 and 20 mg/kg/day doses during a 30-day pilot trial. Perhaps the strongest argument against the above hypothesis arises from our *in vivo* recovery study, which demonstrated that ingested $ClO_2$ is rapidly reduced in the acidic stomach juices to nonoxidizing species (probably $Cl^-$). We were able to recover only 8% of the total oxidizing capacity equivalent of the $ClO_2$, instilled into the animal stomach, after 5 min. of contact time with the gastric contents. This finding is in partial agreement with the radiolabeling studies of Abdel-Rahman et al. (*6*) that demonstrated approximately 80% of the radiochlorine in plasma was in the $Cl^-$ form and about 20% of the $^{36}ClO_2$ was metabolized to $ClO_2^-$ in the rat. A similar distribution of radiochlorine in Abdel-Rahman's study was found in the urine of the animals.

Based on these considerations, it is unlikely that the absorption of a simple chlorine oxide species caused the thyroid effects. An alternative mechanism may be that decrease of dietary iodine absorption in the GI tract due to $ClO_2$ induced mucosal pathology. Abdel-Rahman demonstrated in rats the distribution of radiochlorine after $^{36}ClO_2$ administration was prominently high in the stomach and intestines 72 hr post-administration, indicating the halogen may be covalently attached to the mucosal surface. Inhibition of iodine absorption and alteration of the bioavailability of dietary iodine in the injured intestinal tract would result in progressive iodine deficiency. This was supported by the observation that the T-4 deficiency developed slowly (4 weeks) and progressively in the animals.

Another possibility is that chemical reactions between nutrients and $ClO_2$ give rise to thyroid inhibitory molecules in the GI tract. Such products may interfere with iodine uptake in the thyroglobulin, or they could displace T-4 from binding sites of the carrier, thyroxine-binding globulin. Past toxicologic studies with food ingredients treated with $ClO_2$ (e.g., bleached flour) disclosed no adverse effects on rabbits, monkeys (*17*) and rats (*17*), and on dogs (*17*, *19*). Moran et al. (*20*) identified several modified amino acids in wheat gluten treated with $ClO_2$, e.g., methionine sulfone and mono- and dichlorothyrosine. Other chemical effects were also seen, such as decrease of tocopherol content, etc. Considering the chronology of these studies and the unavailability of methods for thyroid assessment at the time, effects of the $ClO_2$ modified nutrients on thyroid metabolism, if they existed, would have been missed. From Abdel-Rahman's work an additional possibility emerges. About 25% of the radiolabeled chlorine in the liver of rats administered $^{36}ClO_2$ is bound to proteins in the cellular sap, indicating the radiochlorine species may be bound by covalent or by strong hydrogen bonding to these proteins. Such findings could be explained by incorporation of chlorinated amino acids in the protein matrix, or conversely involvement of chlorinated amino acids in thyroid metabolism could be a possible explanation for the observed effect.

Our attempt to find thyroid effects in the serum of human volunteers was unsuccessful. This group consumed only about 1 mg/l. of $ClO_2$ for every liter of drinking water, in which the $ClO_2^-$ and $ClO_3^-$ content did not exceed 5 mg/l. (*11*). The estimated human dose of $ClO_2$ in this study was only about one thousandth ($1 \times 10^{-3}$) of that administered to the monkeys.

Further research is in progress to determine the nature and mechanism of the thyroid inhibitory effect of $ClO_2$.

## Chlorite

Previous findings of Heffernan et al. (*10*) and Moore et al. (*5*) demonstrating oxidative stress induced methemoglobinemia and anemia in rats was verified in this study. Our results show that at most doses $ClO_2^-$ induced a self-compensating oxi-

dative stress in the monkey hematopoiesis. About midway through exposure, a rebound phenomenon occurred in hemoglobin and red cell synthesis. Figures 2 and 3 show male and female hematologic parameters plotted in terms of Delta Standard Deviations (DSD) for comparability. The reticulocytotic response (Fig. 3, solid lines) to $ClO_2^-$ exposure was not accompanied by dose responsive methemoglobinemia.

During $NaClO_2$ exposure a dose-dependent rise of ALT was detected in the monkeys. The extent of enzyme evaluation was subclinical (Table 7) and was not corroborated by concomitant elevation of any other enzyme system or by serum bilirubin. The significance of this observation is not known, but may be associated with accelerated hepatic activity during the transient oxidative hemolytic period.

## Chlorate

The effects of chlorate were similar to those seen during chlorite exposure (Fig. 4 and 5), although the rebound phenomenon was not as clearly discernible.

## Chloramine

At the attainable maximum concentration (100 mg/l.) this compound appeared to have no detectable effect on the hematology of the animals, including red cell GSH content. No evidence of thyroid suppression was detected in the serum.

# Conclusions

The thyroid inhibitory effects of $ClO_2$ ingestion appears to be a significant health endpoint of unknown explanation. The potential for adverse health effects during long-term chronic exposure to low levels of $ClO_2$, specifically to $ClO_2$–modified nutrients, merits further research.

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